

CLAIMS

1. A method of eliciting a T cell response against a T cell epitope in a host mammalian
5 subject, which method comprises:
 - (i) a first immunisation that comprises at least two administrations which are from 1 to 14 days apart to the subject, wherein each administration comprises administering a nucleotide of interest (NOI) encoding the T cell epitope, and optionally
 - (ii) a second immunisation that comprises at least one administration to the subject of (a) a
10 NOI encoding the T cell epitope, or (b) a protein comprising the T cell epitope, wherein the time between
 - the first administration of the first immunisation, and
 - the first administration of the second immunisation,is from 21 to 365 days.
- 15 2. A method according to claim 1 wherein the administrations of the first and/or second immunisation occur over from 2 to 12 days.
3. A method according to claim 1 or 2 wherein in the first and/or second immunisation the NOI or protein is administered from 2 to 10 times.
4. A method according to any one of the preceding claims wherein 2, 3, 4 or more of
20 the administrations of the first and/or second immunisation are from 2 to 6 days apart.
5. A method according to any one of the preceding claims wherein the time between the first administration of the first immunisation and the first administration of the second immunisation is from 50 to 250 days.
- 25 6. A method according to any one of the preceding claims which further comprises a third immunisation that comprises at least one administration to the subject of (a) a NOI encoding the T cell epitope, or (b) a protein comprising the T cell epitope, wherein the time between
 - the first administration of the second immunisation, and

- the first administration of the third immunisation,
is from 10 to 365 days.

7. A method according to claim 6 wherein in the third immunisation:

- the NOI or protein is administered 2 to 5 times, and/or

5 - the administrations are from 2 to 6 days apart, and/or

- the time between the first administration of the second immunisation and the first
administration of the third immunisation is from 50 to 250 days.

10 8. The method according to any one of the preceding claims wherein the NOI
comprises a DNA sequence under the control of a regulatory sequence capable of directing
expression of the DNA sequence in a cell of the subject.

9. The method according to any one of the preceding claims wherein the T cell epitope
is a CD4+ helper T lymphocyte cell epitope and/or CD8+ T lymphocyte (CTL) epitope.

15 10. The method according to any one of the preceding claims wherein one or more of
the administrations of NOI comprise administration of from 0.1 to 2 ug of NOI.

11. The method according to any one of the preceding claims wherein one or more of
the administrations comprises administration to the skin.

20 12. The method according to any one of the preceding claims wherein for at least one
of the administrations of NOI or protein, the NOI or protein is coated on, or incorporated
in, a particle.

13. The method according to claim 12 wherein the particle is administered to the
subject by a particle acceleration device.

25 14. The method according to any one of the preceding claims wherein for at least one
of the administrations of NOI or protein, the NOI or protein is administered as

(i) a pharmaceutical composition comprising a pharmaceutical acceptable carrier, excipient or diluent; or

(ii) a vaccine composition comprising an immunologically acceptable carrier, excipient or diluent; or

5 (iii) an immunotherapeutic composition comprising an immunologically acceptable carrier, excipient or diluent.

15. The method according to any one claims 1 to 13 wherein the NOI or protein is co-administered with an adjuvant or a polynucleotide that is capable of expressing an adjuvant in a cell of the subject; or a method according to claim 14 wherein the composition further
10 comprises an adjuvant or a polynucleotide that is capable of expressing an adjuvant in a cell of the subject.

16. The method according to claim 15 wherein the adjuvant is a non-toxic form of the *E. coli* heat-labile enterotoxin (LT) or the *Vibrio Cholerae* cholera toxin (CT).

17. The method according to claim 15 wherein the adjuvant is the B subunit (LTB) of the LT enterotoxin or B subunit (CTB) of CT cholera toxin.
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18. The method according to any one of the preceding claims wherein the T cell epitope is from a pathogen or from a cancer cell.

19. The method according to any one of the preceding claims wherein the T cell epitope is from HSV, HIV or HPV.

.0 20. The method according to any one of the preceding claims which is carried out to prevent or treat a disease in the subject.

21. The method according to any one of the preceding claims wherein the NOI encodes at least two HSV, HIV or HPV antigens.

22. The method according to claim 21 wherein the NOI encodes an HIV-1 gag protein, or fragment containing a gag epitope thereof, and a second HIV antigen or a fragment encoding an epitope of said second HIV antigen.

23. The method according to claim 22 wherein the second antigen is selected from the group consisting of: Nef, RT or a fragment containing an epitope of Nef or RT.

24. The method according to claim 22 wherein the NOI encodes a combination of antigens selected from the group consisting of:

- Gag (p17,p24), Nef truncate
- Gag (p17,p24) (codon optimised), Nef (truncate)
- 10 - Gag (p17,p24), RT, Nef (truncate)
- Gag (p17,p24), codon optimised RT, Nef (truncate)
- Gag (p17,p24), codon optimised RT, codon optimised Nef truncate;

and/or inactivated codon optimised RT, truncated Nef and p17/p24 portion of the codon optimised gag gene, optionally operatively linked downstream of an Iowa length HCMV promoter + exon 1, and upstream of a rabbit globin poly-adenylation signal.

25. The method according to any one of the preceding claims wherein at least two different NOI's are administered which each encode the same epitope and/or at least two different proteins are administered which comprise the same epitope.

26. An assay for testing the effectiveness of a method of eliciting a T cell response, wherein the method comprises (i) a first immunisation that comprises at least two administrations which are from 1 to 14 days apart to the subject, wherein each administration comprises administering a nucleotide of interest (NOI) encoding a T cell epitope, and optionally

(ii) a second immunisation that comprises at least one administration to the subject of (a) a NOI encoding the T cell epitope, or (b) a protein comprising the T cell epitope, wherein the time between

0 - the first administration of the first immunisation, and

- the first administration of the second immunisation,
is from 21 to 365 days,

wherein the assay comprises carrying out the method on a mammalian subject and then determining the level of activated or memory T cells specific to the epitope in the subject.

- 5 27. An assay according to claim 26 which comprises determining whether
(i) the administrations of the first immunisation all fall within the time period between the first administration of the first immunisation and the decline in the level of activated T cells to basal level, and/or
(ii) the first administration of the second immunisation occurs after the decline in the level
10 of activated T cells to basal level.

28. A kit for carrying out the method or assay of any one of the preceding claims, wherein the kit comprises:

- (i) a NOI as defined in any one of the preceding claims or a composition according to claim
15 14; and
(ii) instructions for administration of the NOI or composition in accordance with the method or assay as defined in any one of the preceding claims.